

factors, such as protein type, pH condition, temperature, acid titrant, NaCl content, spike timing, and post-spike filtration. The DoE showed statistical significance in the predictions of the multivariate models for temperature, but the differences between different temperature conditions have minimal effects on X-MuLV clearance in the study ranges as shown in FIG. 10. FIG. 10 shows scaled estimated LRF for evaluated factors, including NaCl, pH, acid titrant, temperature, protein type (mAb, monoclonal antibody), spike timing and combinations thereof. The retrospective data from industrial operating conditions (Mattila et al.) in the range of from 15° C. to 20° C. showed no statistically significant differences between 15±1° C. and 16+° C. FIG. 10 also shows retrovirus LRF for different temperature conditions based on retrospective industrial data.

Example 8. Evaluate the Factor of Spike Timing

[0106] The statistical DoE was used to evaluate and characterize the effects of a low pH hold step for virus (X-MuLV) inactivation including the evaluation of several factors, such as protein type, pH condition, temperature, acid titrant, NaCl content, spike timing, and post-spike filtration. The spiking timing in the statistically DoE of the present application is an adjust-spike-readjust method or a spike-adjust method. Under the adjust-spike-readjust method, samples are adjusted/titrated to the target pH then spiked with the virus stock having pH 7.2. Timing of the pH hold began at the time of spiking. Due to the observation of pH increases after spiking virus stock, the pH of the samples were readjusted to the target pH prior to being held at the desired temperature for the remainder of the pH hold. Under the spike-adjust method, samples are first spiked with the virus stock solution then adjusted/titrated to the target pH. When the target pH is reached, timing of the pH hold started, and the sample is incubated at the desired temperature.

[0107] The differences in spike timing for two different methods have no meaningful differences on X-MuLV clearance in the study ranges as shown in FIG. 11. FIG. 11 shows scaled estimated LRF for evaluated factors, including NaCl, pH, acid titrant, temperature, protein type (mAb, monoclonal antibody), spike timing and combinations thereof. FIG. 11 also shows the time for spiking/adjusting for two methods.

What is claimed is:

1. A method for purifying a peptide or protein from a sample, the method comprising:

- subjecting the sample to increasing ionic strength by addition of a salt,
 - subjecting the sample to an acidic pH, and
 - subsequently maintaining the sample at the ionic strength condition and the pH condition for at least about 15 minutes to inactivate a quantity of viral particles;
- wherein the sample comprises one or more impurities including the viral particles.

2. The method of claim 1, wherein the quantity of the viral particle inactivation is at least about 3 LRF (logarithmic reduction factor).

3. The method of claim 1, wherein the quantity of the viral particle inactivation is at least about 4 LRF.

4. The method of claim 1, wherein the pH condition of the sample is less than or equal to about pH 3.90.

5. The method of claim 1, wherein the pH condition of the sample is in a range of from about pH 3.60 to about pH 3.90.

6. The method of claim 1, wherein the pH condition of the sample is in a range of from about pH 3.65 to about pH 3.80.

7. The method of claim 1, wherein the peptide or protein is an antibody produced in a host-cell.

8. The method of claim 1, wherein the sample is maintained at the ionic strength condition and the pH condition for at least about 30 minutes to inactivate the quantity of the infectious viral particles.

9. The method of claim 1, wherein the sample is maintained at the ionic strength condition and the pH condition for from about 15 minutes to about 30 minutes to inactivate the quantity of the infectious viral particles.

10. The method of claim 1 further comprising optimizing the ionic strength and the pH condition of the sample for inactivation of the quantity of the infectious viral particles by running a D-Optimal design of experiment.

11. The method of claim 10, wherein the D-Optimal design of experiment evaluates the pH condition of the sample and the ionic strength of the sample and adjusts the pH condition of the sample and the ionic strength of the sample to inactivate a quantity of infectious viral particles.

12. The method of claim 11, wherein the D-Optimal design of experiment further evaluates and adjusts one or more of:

- a conductivity of the sample;
- a type of the peptide or protein;
- a temperature of the sample;
- an acid titrant to adjust the pH condition of the sample;
- a method for spiking the viral particles to the sample; or
- a presence of a post-spike filtration.

13. The method of claim 1, wherein the sample is an eluent from protein A chromatography.

14. The method of claim 1, wherein the ionic strength of the sample is adjusted using an addition of sodium chloride, wherein a concentration of the sodium chloride is in a range of from about 1 mM to about 100 mM.

15. The method of claim 1, wherein the concentration of the sodium chloride is in a range of from about 1 mM to about 500 mM.

16. The method of claim 1, wherein the concentration of the sodium chloride is about 25 mM, about 50 mM, or about 100 mM.

17. The method of claim 1, wherein the pH condition of the sample is adjusted using phosphoric acid or glycine HCl.

18. The method of claim 1, wherein the peptide or protein is an antibody having an IgG1 isotype or having an IgG4 isotype.

19. The method of claim 1, wherein the peptide or protein is a monoclonal antibody or a bispecific antibody.

20. The method of claim 1, wherein the peptide or protein is an antibody, an antibody fragment, a Fab region of an antibody, an antibody-drug conjugate, a fusion protein, a protein pharmaceutical product or a drug.

21. A method of producing a preparation comprising a protein of interest and a reduced amount of viral particles from a sample having the protein of interest and an infectious viral particle, comprising:

- subjecting the sample to a pH of greater than or about 3.6;
- subjecting the sample to an increase in ionic strength condition by addition of a salt to the starting solution; and
- maintaining the sample at the pH and ionic strength condition for an appropriate amount of time to produce